

**SAN JUAN RIVER FISH HEALTH SURVEYS
(1992 - 1999)**

by

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San Juan River Basin Recovery Implementation Program

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November 5, 1999

Prepared for:
San Juan River Biological Committee

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INTRODUCTION:

Fish health surveys on the San Juan River from Farmington, New Mexico, to Clay Hills, Utah, were initiated in October 1992 by Pinetop Fish Health Center (PFHC) personnel. The goal of the survey was to determine the cause of lesions and other abnormalities observed in native and nonnative fish. This report summarizes the fish health findings in San Juan River fish from October 1992 through May 1999.

The investigations focused on the flannelmouth sucker, *Catostomus latipinnis*, as it is the most prevalent fish species sampled and had been the most susceptible species to abnormalities, including lesions. Gaufin, Smith, and Dotson (1960) had reported a similar situation with *C. latipinnis* in the Green River, Wyoming. For these reasons, *C. latipinnis* was used as an indicator species of environmental stressors or diseases. When present, these stressors also may affect the endangered Colorado pikeminnow (squawfish), *Ptychocheilus lucius*, razorback sucker, *Xyrauchen texanus*, and other fish species in the San Juan River.

Fish health sampling included: 1) field observations (e.g., ectoparasite and endoparasite identifications, and noting the incidence of internal and external abnormalities), and 2) subsequent laboratory investigations including viral assays, bacterial identifications, and histological examinations. Fish also were examined for electro-induced damage including spinal deformities and damaged kidney tissue.

MATERIAL AND METHODS:

Fish health personnel accompanied fishery biologists on San Juan River adult monitoring trips from Hogback, New Mexico, to Mexican Hat, Utah. Additional trips were made to sample fish from Mexican Hat to Clay Hills, Utah, and Farmington to Hogback, New Mexico. All fish were collected using rafts mounted with electrofishing apparatus. All fish species with abnormalities were noted, including but not limited to: bluehead sucker (*Catostomus discobolus*), flannelmouth sucker (*C. latipinnis*), razorback sucker (*X. texanus*), channel catfish (*Ictalurus punctatus*), Colorado pikeminnow (*P. lucius*), roundtail chub (*Gila robusta*), common carp (*Cyprinus carpio*), red shiner (*Cyprinella lutrensis*), fathead minnow (*Pimephales promelas*), and speckled dace (*Rhinichthys osculus*). Fishery biologists noted and saved fish with lesions, tumors, and missing or deformed eyes for subsequent observations by fish health personnel. For purposes of this report, a lesion is defined as an abnormal change in skin structure due to injury and/or infectious disease. Disease, defined as lack of ease or as a stressed condition, encompasses all abnormalities in this report. Other stressors including leeches and obvious predator-inflicted wounds were noted by biologists, but were not considered abnormalities.

Field notes were obtained from the fishery biologists and analyzed for the number of each taxa collected and what types of abnormalities were observed. This provided a river mile by river mile accounting of observations for a large number of fish.

In May 1994, fish health personnel initiated disease “inspections” of randomly selected *C. latipinnis* from all electrofishing boats. The purpose of the inspections were 1) to supplement disease samples collected by fishery biologists, 2) to ascertain health status of apparently healthy and moribund fish in regular intervals throughout the sampling region, and 3) to use *C. latipinnis* as an indicator species of potential environmental hazards affecting other animal and plant life in the San Juan River. Inspections

were performed ~~DRAFT~~ Random five-fish sample every designated mile (every fifth river mile).

Fish health personnel performed complete necropsies on all inspection fish, as well as fish with abnormalities saved by other biologists. Necropsy included: external and internal inspection for gross pathology (lesions, organ deformities, unusual color, excess peritoneal fluid, etc.), bacterial sampling from kidneys and lesions using sterile techniques (disinfecting the leading edge of lesions with 70 percent isopropyl alcohol), and chemical fixation of various organs for histopathological examination. Electrical power for light microscopic observation of skin and gill wet-mount preparations on selected fish for ectoparasites was provided by a backpack electrofishing generator.

Bacterial samples were isolated on one of two standard bacterial media: brain-heart infusion agar (BHIA) or trypticase soy agar (TSA). Bacterial isolates were identified in the laboratory using the Minitek™ classification system. A fish was considered bacteremia-positive when bacteria was cultured from kidney tissue. Bacteremia was defined as presence of bacteria in the blood; this is considered a disease state because blood and kidneys are sterile in healthy fish.

Tissue fixation for histology was performed in the field using Bouin's fixative or 10 percent formalin for 48 hours with subsequent transfer to 70 percent isopropyl alcohol. Preserved tissues were sent to the National Fish Health Laboratory, Biological Resources Division (USGS), Kearneysville, West Virginia, for histopathological examination. Potential electrofishing-induced damage was measured by noting gross physiological trauma to kidneys and by radiographing (X-ray) randomly collected fish to identify vertebral damage. Asian tapeworms, *Bothriocephalus acheilognathi*, were observed by dissecting fish gastrointestinal tracts using stereo microscopes in the field. Virology tissues were stored in minimal essential medium (MEM) at 4° C and returned to the PFHC for viral tissue culture assays.

RESULTS:

Fish species with abnormalities: Even though *C. latipinnis* comprised between 50-60 percent of all fish sampled during each fish survey, it accounted for 70-90 percent of all abnormalities observed. Depending on the survey, *C. carpio*, *C. discobolus*, or *I. punctatus* ranged from 1 percent to 15 percent of the abnormalities.

Abnormality types: Lesions comprised a majority of the abnormality types, but missing or deformed eyes, scoliosis or lordosis, and a few tumors were found during the fourteen sampling periods. Since the May 1996 survey, there has been a decrease of lesions observed that continued through May 1999. Most of the abnormalities noted in the latter period were damaged eye tissue. Spinal deformities continued to decrease each sampling period.

Percentage of fish with abnormalities/Fish health inspection data: Randomly selected *C. latipinnis* (inspection fish) from each designated mile are represented in Table 1. Each of these fish represents a complete necropsy procedure and all abnormalities were noted by fish health biologists.

Table 1. San Juan River *C. latipinnis* fish health biologists' inspection data for abnormalities, 10/94 - 5/99:

Date	# of fish sampled	# of fish with abnormalities	percent of fish with abnormalities
10/94	95	5	5
5/95	99	8	8
10/95	100	5	5
5/96	100	19	19
10/96	100	4	4
5/97	100	1	1
10/97	110	1	0.9
5/98	80	2	2.5
5/99	80	3	3.8

During the May 1996 survey, the health of the sucker populations were in poor condition compared to all other survey periods. Fish health biologists not only sampled the randomly selected *C. latipinnis*, but also necropsied an additional 231 fish. These fish were mostly suckers and were selected due to abnormalities. Of these 331 fish examined, 145 had lesions. More fish with abnormalities were observed during the 1993 survey than during any other survey, with biologists saving abnormal fish for the fish health biologists. During 1997-1999, fish health biologists have observed only an occasional lesion, with no concentration of abnormalities in a specific area of the river.

Fishery biologist data: Fishery biologist data included all fish species collected and abnormalities observed. Utilizing the data for *C. latipinnis* and *C. discobolus*, survey periods were compared (Table 2). This provided a large number of suckers in the data base and changes in frequencies of abnormalities from each river mile provided trends of probable problem areas of the San Juan River. The discrepancy in values between fishery biologist and fish health biologist data is explained by the fish health biologists' ability to observe pathology more carefully with more time allotted to fish health. The lower fishery biologist abnormality incidence values may be attributed in part by the inclusion of *C. discobolus* values which lowered the overall abnormality incidence rate. However, the same trend in abnormality incidences was observed in both data sets from each type of survey.

Table 2. Six-year review of fishery biologists' San Juan river *C. latipinnis* and *C. discobolus* abnormality data (10/91 - 10/97):

Date	# of fish sampled	% of fish with abnormalities	% of abnormalities that are lesions
10/91	1606	2.6	54
6/92	3018	3.6	65
10/92	3413	0.3	30
10/93	2959	0.4	55
5/94	1878	3.0	74
10/94	9524	0.6	30
5/95	1266	0.1	25
10/95	2261	0.8	25
5/96	2557	3.4	72
10/96	2888	1.6	33
5/97	5270	0.4	19
10/97	7440	0.3	32

Abnormality incidences versus river mile (RM)/Fish health inspection data: During years of high abnormalities, most were found in sections RM 121-156 and RM 76.

Fishery biologist data: Most of the abnormalities observed by fishery biologists consistently were found on fish in RM 126-156. As with fish health inspection data, fewer abnormalities were found in the Fall (October) compared to Spring (May) surveys. Few abnormalities were found from Lake Powell (RM 0) to the head of the canyon (RM 78). However, bite marks caused by fish predators were common. From the head of the canyon to Farmington, New Mexico (RM 178), wounds from bird predators were also common. Both of these wound types were easily distinguished from lesions.

Bacterial isolates of San Juan River fish: Bacterial isolates from all survey trips are identified in Table 3. Bacteremia, as indicated by bacterial growth from kidney tissue tended to follow the abnormality trends on each of the surveys.

Table 3. Bacteria isolated from San Juan River fish, 1994-1999.

Taxa	lesion	kidney
<i>Acinebacter anitrans</i>		X
<i>A. lwoffii</i>	X	X
<i>Actinobacillus lignieresii</i>	X	
<i>Aeromonas</i> sp.	X	X
<i>Aeromonas hydrophila</i>	X	X
<i>Citrobacter freundii</i>		X
<i>Enterobacter agglomerans</i>	X	
<i>E. cloacae</i>		X
<i>E. sakazaki</i>	X	
<i>Flavobacterium</i> sp.	X	
<i>F. meningosepticum</i>		X
<i>Hafnia alveri</i>	X	
<i>Klebsiella arizonae</i>		X
<i>K. pneumoniae</i>		X
<i>Pleisiomonas shigelloides</i>	X	X
<i>Proteus mirabilis</i>	X	
<i>P. vulgaris</i>	X	
<i>Pseudomonas</i> sp.	X	X
<i>P. aeruginosa</i>	X	
<i>P. cepacia</i>	X	X
<i>P. fluorescens</i>	X	
<i>P. maltophilia</i>	X	X
<i>P. multifili</i>		X
<i>P. pseudoalcaligenes</i>	X	X
<i>P. putrefaciens</i>	X	X
<i>P. stutzeri</i>		X

<i>P. vesicularis</i>		X
<i>Salmonella arizonae</i>	X	
<i>Serratia liquifaciens</i>	X	
<i>S. odorifera</i>	X	X
<i>S. rubidaea</i>	X	X
<i>Shigella</i> sp.	X	X
<i>Staphylococcus</i> sp.	X	X
<i>Streptococcus</i> sp.		X
<i>Vibrio alginolyticus</i>	X	X
<i>V. cholerae</i> (non-human)	X	
<i>V. fluvialis</i>	X	X
<i>Yersinia enterocolitica</i>	X	
<i>Y. pseudotuberculosis</i>	X	
Gram positive rods	X	X

Electrofishing damage: Damaged kidney tissue was noted in fish necropsies and spinal deformities were noted from both fishery and fish health biologists' observations. Randomly collected *C. latipinnis* from each electrofishing raft were radiographed on vertical and lateral planes and analyzed for spinal damage of any type (Alcumbrac 1994, personal communication). Both compression and step-down fractures were found in less than 10 percent of the fish. Of the three rafts surveyed, there was no correlation with manufacture of the unit; however, those rafts with a larger cathode surface area had the least amount of spinal damage to fish. Since the radiograph survey was completed, rafts have been modified, which probably has accounted for fewer spinal abnormalities.

Asian Tapeworm and other parasites: Asian tapeworms were first detected in *C. carpio* in the San Juan River near Bluff, Utah (RM 82) in October 1994 (Landye and McCasland 1997). The invasion of this tapeworm to other sections of the river and cyprinid species was documented in subsequent surveys from Mexican Hat (RM 52.8) to Fruitland Diversion, New Mexico (RM 166). This included infections found in older stocked *P. lucius*, but not young of the year stocked fish. No Asian tapeworms have been found in other families of fish in the San Juan River.

The following endo- and ectoparasites were observed during at least one fish health survey. The species name following the parasite name indicates the fish from which the parasites were detected: *Ambiphyra* (*I. punctatus*), *Apiosoma* (*I. punctatus*), *Trichodina* (*Micropterus salmoides*, *I. punctatus*, *C. carpio*, *C. latipinnis*, and *C. discobolus*), *Tetrahymena* (*C. latipinnis*), *Henneguya* (*I. punctatus*), *Gyrodactylus* (*C. carpio*, *C. latipinnis*, and *C. discobolus*), *Ligictaluridus* (*I. punctatus*), *Hunterella* (*C. latipinnis*), *Corallobothrium fimbriatum* (*I. punctatus*), *B. acheilognathi* (*C. carpio*, *C. lutrensis*, *R. osculus*, and *P. lucius*), *Myzobdella* (*I. punctatus*), and *Lernaea* (*C. latipinnis*).

Ichthyophthirius (“Ich”) was not detected during main river fish health surveys, but Ich positive samples from backwater areas were examined.

Rainbow and brown trout were occasionally sampled and cranial tissue was taken for plankton centrifuge method of analysis for *Myxobolus cerebralis*, the causative agent for whirling disease. Even though this area is not suitable for salmonid populations, the cold spring water runoffs allowed for a few trout to be present. A sample of the seasonal trout population was taken, but no *M. cerebralis* spores were detected.

Viruses: All viral assays from San Juan River fish were negative from samples taken from May 1994 to May 1999.

Histopathology findings: Lesion samples were collected and shipped to Dr. Vicki Blazer, National Fish Health Laboratory, Kearneysville, West Virginia, for histological analysis. Dr. Blazer presented three potential explanations for the lesions: “1) some mineral deposit, possibly with sharp projections, gets into the dermis of bottom dwelling fish and causes the reaction; 2) A metabolic disorder, possibly in response to some contaminant, which causes the body to produce the material or deposit it, abnormally in the dermis, which lead to a reaction to it; and 3) Some interaction of a contaminant with Ultra Violet radiation which leads to skin damage.”(Blazer 1996, personal communication). Dr. Blazer’s opinion on the presence of “masses of bacteria” in the eroded epidermis and associated necrotic muscle is that they are secondary invaders, which concurs with the analysis.

DISCUSSION:

Flannelmouth suckers as an indicator species: *C. latipinnis* remained the best choice as an indicator species for environmental problems on the San Juan River throughout the study. This species has the highest proportion of abnormalities (in excess of 80 percent) than any other fish species sampled, but was only about 60 percent of all fish sampled. This is consistent with the abnormalities and fish species observed by Gaufin, Smith, and Dotson (1960) in the Green River.

Cause of lesions and other abnormalities: We hypothesize that bacteria isolated from fish lesions are secondary invaders—they are opportunistic organisms that cause lesions after initial trauma due to contaminants, unsuccessful bird or fish predation, etc. The National Fish Health Laboratory concurs that the bacteria are secondary. Dr. Blazer also proposes (in two of her three explanations for the cause of lesions) that a contaminant was present which initiates the epidermal necrosis. Walker (1992) in a preliminary investigation on lesions from suckers in the Animas River, a tributary to the San Juan River, thought that the cause was a type of *Aeromonas salmonicida* bacterium. Gaufin, Smith, and Dotson (1960) noted that most of the flannelmouth suckers with lesions were just downstream of Green River, Wyoming, where municipal sewage and oil discharge was taking place.

Histological samples consistently have shown an unknown material in micro quantities to be present in lesion sections. Herman and Lemm (1990) found this possible putative autoimmune reaction in striped bass. Until a micro-probe is available to analyze this material, the probable cause of these fish lesions will remain unknown. From all the fish health samples taken during this investigation, we can eliminate

viral, bacterial, or parasitic agents as the primary cause of lesions in San Juan River fish.

Gross pathology data from inspection fish and field notes of both fish health and fishery biologists demonstrated a decreasing abnormality incidence from 1993 to 1999. However, lesions increased in May 1996 to levels resembling the disturbing May 1994 and June 1992 levels. It is unlikely that these spikes of lesions can be attributed solely to spawning stress that occurs annually.

Bacterial isolates of San Juan River fish: Of all the bacterial species isolated from all fish sampled, none are known primary fish pathogens, although *Aeromonas hydrophila*, the causative agent of Motile Aeromonas Septicemia (MAS), usually presents itself after the fish has been stressed. However, most would be considered opportunistic or secondary fish pathogens. These taxa would affect fish that have been compromised by lesions, reproductive stress, and predator wounds. Fish carrying these bacteria are not an immediate threat to humans in contact with the San Juan River. Given the number of bacterial taxa present in the system, and new evidence that species such as *Pseudomonas aeruginosa* can affect both plants and animals, humans with already compromised health conditions should take precautions with water contact between Bluff, Utah, and Farmington, New Mexico, where most of these bacteria were recorded.

Electrofishing damage: Possible electrofishing damage was noted by observation of spinal deformities in fishery biologists data and damaged kidney tissue during fish health surveys. The radiographs of randomly collected “healthy” *C. latipinnis*, demonstrated that most of the damage was in the form of compression fractures while step-down fractures were poorly represented. These compression fractures account for many of the spinal deformities found in the flannelmouth suckers. Modifications to cathodes of the electrofishing units during the study probably resulted in the reduction in spinal deformities noted during the course of the study. There was no correlation with electrofishing damage and manufacture of equipment.

Asian tapeworm and other parasites: Asian tapeworms, *Bothriocephalus acheilognathi*, will remain in the San Juan River as long as susceptible hosts are present. *B. acheilognathi* will pose a threat to native speckled dace, roundtail chub, and young Colorado pikeminnow. “Ich,” *Ichthyophthirius multifiliis*, is a potential problem to young of the year fish in backwaters during the warmer water temperatures. Leeches may provide areas that secondary bacteria can invade and young forms of *Lernaea* might have an effect on fish in backwaters during periods of warmer water. All other parasites should have little or no effect on fish in the future unless water quality deteriorates.

Virus: Since no viral evidence was found in San Juan River fishes, they are currently not a stress factor in fish populations.

Severity of disease in the San Juan River and Summary: Since June 1992, San Juan River fish health data suggests that abnormality incidence in all fish species is low with the exception of “spikes” of lesions and other disease signs during the Spring hydrograph. River sections with the highest abnormality incidence and bacteremia constantly have been miles 136-156. Because of the inconsistency of spikes of lesions from year to year, it would suggest that the source might be triggered by monsoon cyclonic storm activity from the previous summer or occasional releases from a yet unknown source.

Lesions remain the most appropriate abnormality for use as an indicator of stressful environmental conditions and resulting fish disease. The reason for this is that the years when the percentage of fish with abnormalities is high, the percentage of abnormalities that are lesions also is high. Thus, incidences of lesions in *C. latipinnis* will continue to be a useful tool as the primary indicator of fish health in the San Juan River.

Any future investigations should integrate histopathological methods with the use of a micro probe (when developed) or some of the new techniques utilizing proteins as a method to detect fish stress.

Conclusions:

- Fish pathogens are not the primary cause of the majority of lesions observed on San Juan River fish.
- Asian tapeworm, *B. acheilognathi*, was discovered within a few months of its initial introduction, and its infection of cyprinids was documented both upstream and downstream as it spread.
- A large number of bacterial species were present on and in San Juan River fish. Many of these taxa have the potential of secondary infections in both fish and mammals.
- Reconfiguration of electrofishing equipment (cathodes) can reduce the amount of injury to fish.
- Flannelmouth sucker, *C. latipinnis*, remains the best species for monitoring.
- Future research on fish lesions should concentrate on the development of a nano probe to analyze the unknown material found in histopathological samples of lesions.

EXECUTIVE SUMMARY:

Fish health surveys on the San Juan River from Farmington, New Mexico to Clay Hills, Utah were initiated in October 1992 by Pinetop Health Center personnel. The goal of the survey was to determine the cause of lesions and other abnormalities observed in native and nonnative fish. This report summarizes the fish health findings from October 1992 through May 1999.

The investigation focused on the flannelmouth sucker, *Catostomus latipinnis*, as it is the most common fish species sampled and had been the most susceptible species to abnormalities, including lesions. Since this section of the San Juan River was part of the recovery area of the endangered Colorado pikeminnow (squawfish), *Ptychocheilus lucius*, and threatened razorback sucker, *Xyrauchen texanus*, concern about these abnormalities was high.

Fish health sampling included both field observations and laboratory investigations. Fish also were examined for electro-induced injury.

As a result of the fish health survey, the introduced Asian tapeworm was discovered, a large number of bacterial species were identified, and modifications in electrofishing equipment were made to minimize damage to fish in the future. The exact cause of the lesions was not determined, but parasitic, bacterial, and viral agents have been eliminated. Future investigations should be undertaken when an appropriate

nano probe is developed for the unidentified material found in histopathological examinations.

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